

Test Report
2014-TBR-000005

Silver ion sterilizer

Sterilization test

Head of the Korea Testing and Research Institute

Test Overview

Test item: Sterilization test.

Issuance No.: 2014-TBR-000005.

Name of sample: Silver ion sterilizer.

Objective of test: To measure the sterilizing effect of the sample, sterilization tests were conducted for *E. coli*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *S. flexneri*, *B. cereus*, *B. subtilis* and *L. monocytogenes*.

Test method: Provided by client – this test was conducted according to the test method presented by the client.

Client

Name: CNL.

Address: 1 Yeonsedaegil, Heungeop-myeon, Wonju-si, Gangwon-do, Korea
(304 Disabled Business Incubation Center)

Representative: Kim Gyeongsu.

Contact: Tel. 033-764-2116, Fax. 033-764-2117.

Testing laboratory

Name: Korea Testing and Research Institute

Address: 196 Aegibongro, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea.

Contact: Tel. 031-999-3193, Fax. 031-999-3005.

Test period: September 17 2014 – November 14 2014.

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1. Summary

To investigate the sterilization effect of the sample (silver ion sterilizer), sterilization tests for strains requested by the client, i.e., *E. coli*, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *S. flexneri*, *B. cereus*, *B. subtilis* and *L. monocytogenes*, were conducted in accordance with a test method presented by the client.

The results of the measurement of the sterilization effect of the sample (silver ion sterilizer) were: for *E. coli*, 89.2% at 30 min, 99.8% at 2 hr and more than 99.9% at 24 hr; for *S. aureus*, 58.6% at 30 min, 95.9% at 2 hr and more than 99.9% at 24 hr; for *S. typhimurium*, 96.6% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *P. aeruginosa*, 42.0% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *S. flexneri*, 26.7% at 30 min, 60.0% at 2 hr and more than 99.9% at 24 hr; for *B. cereus*, 89.1% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *B. subtilis*, 89.1% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; and for *L. monocytogenes*, 43.1% at 30 min, 94.4% at 2 hr and more than 99.9% at 24 hr;

2. Equipment and materials

2.1 Testing equipment

Autoclave	(Koatech, Korea)
Dry oven	(Jisico, Korea)
Water bath	(Polyscience, USA)
Incubator	(Mettler, Germany)
pH meter	(Thermo Orion, USA)
Stop watch	(Time Art, Japan)
Vortex mixer	(Thermolyne, USA)
Container	(Iwaki Pyrex, Japan)
Sterile pipette	(FALCON, USA)
Petri dish	(Green Cross Medical Corp., Korea)
Volumetric flask	(Myung Sung, Korea)
Mechanical shaker	(Jisico, Korea)
Clean bench	(Sugong Tech, Korea)
Colony counter	(Deokwoo Science, Korea)

2.2 Testing materials

- 1) Test strains: *Escherichia coli* ATCC 25922
Staphylococcus aureus ATCC 6538
Salmonella typhimurium ATCC 13311
Pseudomonas aeruginosa ATCC 27853
Shigella flexneri ATCC 12022
Bacillus cereus ATCC 14579
Bacillus subtilis ATCC 6633
Listeria monocytogenes ATCC 19111

- 2) Media and reagents

- A. Brain heart infusion (DIFCO, USA)
- B. Brain heart infusion agar (DIFCO, USA)
- C. Tryptic soy agar (DIFCO, USA)
- D. D/E neutralizing broth (DIFCO, USA)
- E. Physiological saline (self produced)

3. Test method

3.1 Test method

The strains used for the tests were cultured in liquid media and diluted to an initial bacterial population of $(1 \sim 9) \times 10^5$ CFU/mL.

The sample (silver ion sterilizer) was set to Stage 4 and a filter connected to tap water was connected to the sample. The tap was adjusted to 2 L/min as indicated on the LED monitor attached to the sample, the solution produced from which were used as the test solution. 0.2 mL of each test strain was inoculated to 20 mL of the test solution (undiluted), mixed and left at room temperature. To determine sterilization reduction compared to the initial bacterial population, bacterial populations were counted 30 min, 2 hr, and 24 hr. The initial bacterial population was determined by inoculating the same as above to a solution passed through the filter connected to tap water and measuring its bacterial population.

In the initial dilution stage of all experiments, D/E neutralizing broth (DIFCO) was used for neutralization. If bacteria proliferated in the media, the bacterial population was calculated by multiplying the number of bacteria in media by the dilution factor. If bacterial did not proliferate, it was indicated less than 10 (< 10) by multiplying the dilution factor during the neutralization stage. In all stages, bacterial populations were measured using TSA (see 2.2), live bacterial levels were measured according to 3.2 [Formula 1], and sterilization reduction rates were measured according to [Formula 2].

3.2 Result calculation

1) Calculation of live bacteria level: [Formula 1] $N = C \times D \times V$

N: Live bacteria level

C: Number of colonies (average of colonies in two media)

D: Dilution factor (dilution factor of diluted solution)

2) Calculation of reduction rate (%): [Formula 2] $R (\%) = [(A - B) / A] \times 100$

R: Reduction rate

A: Initial bacterial population

B: Bacterial population at certain time point

4. Results

4.1 Sterilization test for *E. coli*

From initial bacterial population of $[5.0 \times 10^5 \text{ CFU/mL}]$ to $[5.4 \times 10^4 \text{ CFU/mL}]$ at 30 min, $[8.1 \times 10^2 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.2 Sterilization test for *S. aureus*

From initial bacterial population of $[2.9 \times 10^5 \text{ CFU/mL}]$ to $[1.2 \times 10^5 \text{ CFU/mL}]$ at 30 min, $[1.2 \times 10^4 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.3 Sterilization test for *S. typhimurium*

From initial bacterial population of $[9.6 \times 10^5 \text{ CFU/mL}]$ to $[3.3 \times 10^4 \text{ CFU/mL}]$ at 30 min, $[5.5 \times 10^2 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.4 Sterilization test for *P. aeruginosa*

From initial bacterial population of $[8.1 \times 10^5 \text{ CFU/mL}]$ to $[4.7 \times 10^5 \text{ CFU/mL}]$ at 30 min, $[5.9 \times 10^2 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.5 Sterilization test for *S. flexneri*

From initial bacterial population of $[4.5 \times 10^5 \text{ CFU/mL}]$ to $[3.3 \times 10^5 \text{ CFU/mL}]$ at 30 min, $[1.8 \times 10^5 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.6 Sterilization test for *B. cereus*

From initial bacterial population of $[6.0 \times 10^5 \text{ CFU/mL}]$ to $[5.2 \times 10^4 \text{ CFU/mL}]$ at 30 min, $[6.0 \times 10^3 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.7 Sterilization test for *B. subtilis*

From initial bacterial population of $[1.1 \times 10^5 \text{ CFU/mL}]$ to $[1.2 \times 10^4 \text{ CFU/mL}]$ at 30 min, $[1.1 \times 10^2 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

4.8 Sterilization test for *L. monocytogenes*

From initial bacterial population of $[1.6 \times 10^5 \text{ CFU/mL}]$ to $[9.1 \times 10^4 \text{ CFU/mL}]$ at 30 min, $[9.0 \times 10^3 \text{ CFU/mL}]$ at 2 hr, and $[<10 \text{ CFU/mL}]$ at 24 hr.

5. Discussion and conclusion

The results of the measurement of the sterilization effect of the sample (silver ion sterilizer) were: for *E. coli*, 89.2% at 30 min, 99.8% at 2 hr and more than 99.9% at 24 hr; for *S. aureus*, 58.6% at 30 min, 95.9% at 2 hr and more than 99.9% at 24 hr; for *S. typhimurium*, 96.6% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *P. aeruginosa*, 42.0% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *S. flexneri*, 26.7% at 30 min, 60.0% at 2 hr and more than 99.9% at 24 hr; for *B. cereus*, 89.1% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; for *B. subtilis*, 89.1% at 30 min, 99.9% at 2 hr and more than 99.9% at 24 hr; and for *L. monocytogenes*, 43.1% at 30 min, 94.4% at 2 hr and more than 99.9% at 24 hr;

6. References

- 6.1 Test method provided by the client.
- 6.2 *Clinical Microbiology*, Chunggu, 2003.
- 6.3 *Brock Biology of Microorganisms* (11th edition), World Science, 2006.
- 6.4 *Medical Microbiology*, Koryo Medicine, 2002.

7. Table

Table 3. Summary of sterilization tests

(Unit: CFU/mL)

Test strain	Initial	30 min	2 hr	24 hr
<i>E. coli</i>	5.0 x 10 ⁵	5.4 x 10 ⁴ (89.2%)	8.1 x 10 ² (99.8%)	< 10 (More than 99.9%)
<i>S. aureus</i>	2.9 x 10 ⁵	1.2 x 10 ⁵ (58.6%)	1.2 x 10 ⁴ (95.9%)	< 10 (More than 99.9%)
<i>S. typhimurium</i>	9.6 x 10 ⁵	3.3 x 10 ⁴ (96.6%)	5.5 x 10 ² (99.9%)	< 10 (More than 99.9%)
<i>P. aeruginosa</i>	8.1 x 10 ⁵	4.7 x 10 ⁵ (42.0%)	5.9 x 10 ² (99.9%)	< 10 (More than 99.9%)
<i>S. flexneri</i>	4.5 x 10 ⁵	3.3 x 10 ⁵ (26.7%)	1.8 x 10 ⁵ (60.0%)	< 10 (More than 99.9%)
<i>B. cereus</i>	6.0 x 10 ⁵	5.2 x 10 ⁴ (91.3%)	6.0 x 10 ³ (99.0%)	< 10 (More than 99.9%)
<i>B. subtilis</i>	1.1 x 10 ⁵	1.2 x 10 ⁴ (89.1%)	1.1 x 10 ² (99.9%)	< 10 (More than 99.9%)
<i>L. monocytogenes.</i>	1.6 x 10 ⁵	9.1 x 10 ⁴ (43.1%)	9.0 x 10 ³ (94.4%)	< 10 (More than 99.9%)

* Reduction rate (%) = (A – B) / A x 100

Where, A = Initial bacterial population

B: Bacterial population at certain time point

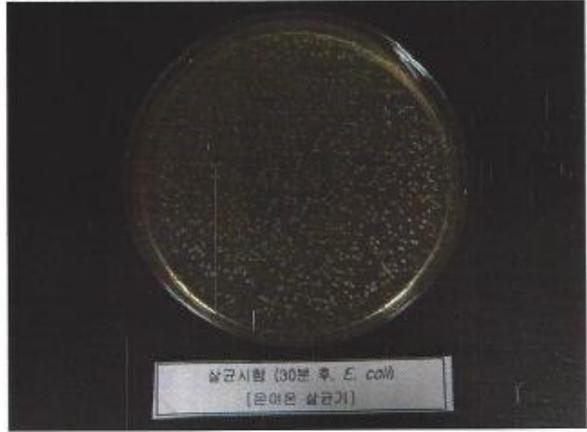
8. Attachments

8.1 Images of test results



살균시험 (초기, *E. coli*)
[은이온 살균기]

Sterilization test (initial, *E. coli*)
[Silver ion sterilizer]



살균시험 (30분 후, *E. coli*)
[은이온 살균기]

Sterilization test (30 min, *E. coli*)
[Silver ion sterilizer]



살균시험 (2시간 후, *E. coli*)
[은이온 살균기]

Sterilization test (2 hr, *E. coli*)
[Silver ion sterilizer]



살균시험 (24시간 후, *E. coli*)
[은이온 살균기]

Sterilization test (24 hr, *E. coli*)
[Silver ion sterilizer]



Sterilization test (initial, *S. aureus*)
[Silver ion sterilizer]



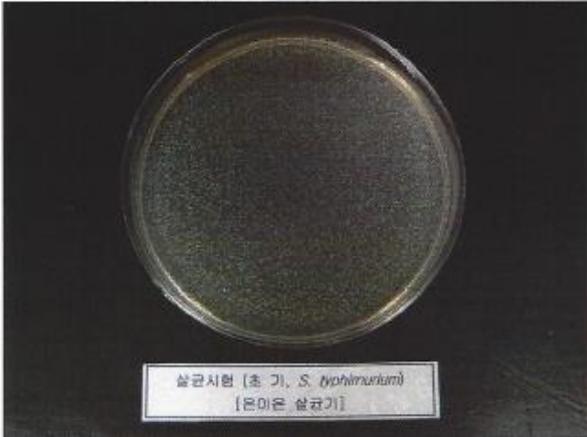
Sterilization test (30 min, *S. aureus*)
[Silver ion sterilizer]



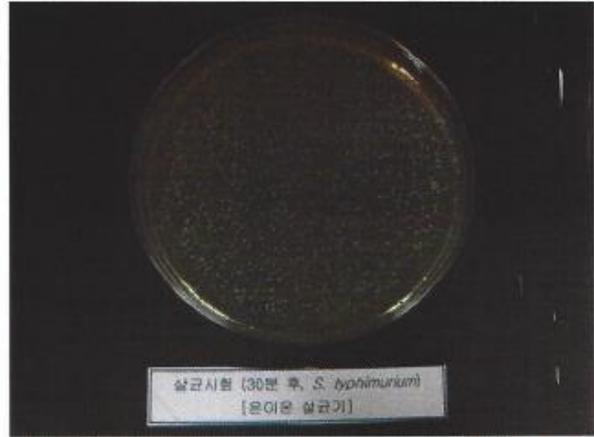
Sterilization test (2 hr, *S. aureus*)
[Silver ion sterilizer]



Sterilization test (24 hr, *S. aureus*)
[Silver ion sterilizer]



Sterilization test (initial, *S. typhimurium*)
[Silver ion sterilizer]



Sterilization test (30 min, *S. typhimurium*)
[Silver ion sterilizer]



Sterilization test (2 hr, *S. typhimurium*)
[Silver ion sterilizer]

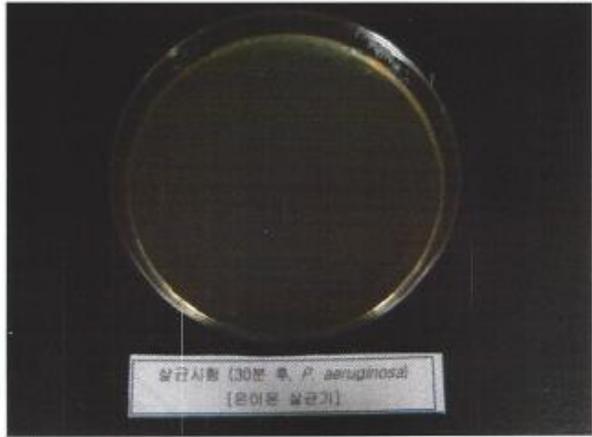


Sterilization test (24 hr, *S. typhimurium*)
[Silver ion sterilizer]



살균시험 (초기, *P. aeruginosa*)
[은이온 살균기]

Sterilization test (initial, *P. aeruginosa*)
[Silver ion sterilizer]



살균시험 (30분 후, *P. aeruginosa*)
[은이온 살균기]

Sterilization test (30 min, *P. aeruginosa*)
[Silver ion sterilizer]



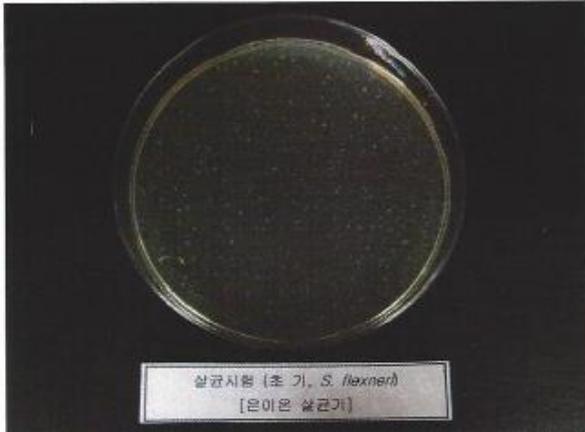
살균시험 (2시간 후, *P. aeruginosa*)
[은이온 살균기]

Sterilization test (2 hr, *P. aeruginosa*)
[Silver ion sterilizer]



살균시험 (24시간 후, *P. aeruginosa*)
[은이온 살균기]

Sterilization test (24 hr, *P. aeruginosa*)
[Silver ion sterilizer]



살균시험 (초기, *S. flexneri*)
[은이온 살균기]

Sterilization test (initial, *S. flexneri*)
[Silver ion sterilizer]



살균시험 (30분 후, *S. flexneri*)
[은이온 살균기]

Sterilization test (30 min, *S. flexneri*)
[Silver ion sterilizer]



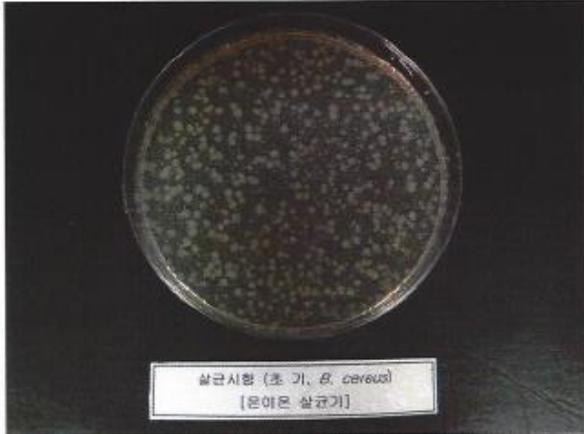
살균시험 (2시간 후, *S. flexneri*)
[은이온 살균기]

Sterilization test (2 hr, *S. flexneri*)
[Silver ion sterilizer]



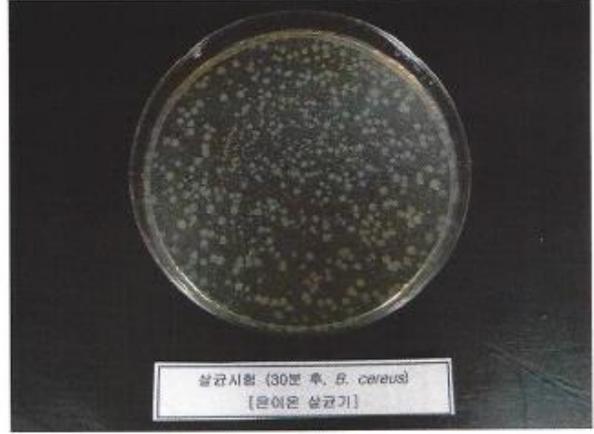
살균시험 (24시간 후, *S. flexneri*)
[은이온 살균기]

Sterilization test (24 hr, *S. flexneri*)
[Silver ion sterilizer]



살균시험 (초기, *B. cereus*)
[은이온 살균기]

Sterilization test (initial, *B.cereus*)
[Silver ion sterilizer]



살균시험 (30분 후, *B. cereus*)
[은이온 살균기]

Sterilization test (30 min, *B.cereus*)
[Silver ion sterilizer]



살균시험 (2시간 후, *B. cereus*)
[은이온 살균기]

Sterilization test (2 hr, *B.cereus*)
[Silver ion sterilizer]



살균시험 (24시간 후, *B. cereus*)
[은이온 살균기]

Sterilization test (24 hr, *B.cereus*)
[Silver ion sterilizer]



Sterilization test (initial, *B. subtilis*)
[Silver ion sterilizer]



Sterilization test (30 min, *B. subtilis*)
[Silver ion sterilizer]



Sterilization test (2 hr, *B. subtilis*)
[Silver ion sterilizer]



Sterilization test (24 hr, *B. subtilis*)
[Silver ion sterilizer]



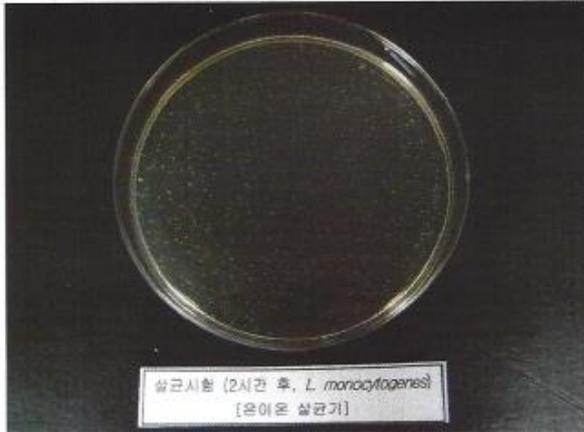
살균시험 (초기, *L. monocytogenes*)
[은이온 살균기]

Sterilization test (initial, *L. monocytogenes*)
[Silver ion sterilizer]



살균시험 (30분 후, *L. monocytogenes*)
[은이온 살균기]

Sterilization test (30 min, *L. monocytogenes*)
[Silver ion sterilizer]



살균시험 (2시간 후, *L. monocytogenes*)
[은이온 살균기]

Sterilization test (2 hr, *L. monocytogenes*)
[Silver ion sterilizer]



살균시험 (24시간 후, *L. monocytogenes*)
[은이온 살균기]

Sterilization test (24 hr, *L. monocytogenes*)
[Silver ion sterilizer]

TEST REPORT

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Report No.: TBR-000005 Date received: Sep 17 2014
 Representative: Kim Gyeongsu Test completed: Nov 14 2014
 Name of company: CNL
 Address: 1 Yeonsedaegil, Heungeop-myeon, Wonju-si, Gangwon-do, Korea
 (304 Disabled Business Incubation Center)

Name of sample: Silver ion sterilizer

Test Results				
Test item	Unit	Sample category	Result	Test method
Sterilization test (E. coli)	CFU/mL	Initial	5.0 x 10 ⁵	Provided by client
Sterilization test (E. coli)	CFU/mL	30 min	5.4 x 10 ⁴ (89.2%)	Provided by client
Sterilization test (E. coli)	CFU/mL	2 hr	8.1 x 10 ² (99.8%)	Provided by client
Sterilization test (E. coli)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client
Sterilization test (S. aureus)	CFU/mL	Initial	2.9 x 10 ⁵	Provided by client
Sterilization test (S. aureus)	CFU/mL	30 min	1.2 x 10 ⁵ (58.6%)	Provided by client
Sterilization test (S. aureus)	CFU/mL	2 hr	1.2 x 10 ⁴ (95.9%)	Provided by client
Sterilization test (S. aureus)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client
Sterilization test (S. typhimurium)	CFU/mL	Initial	9.6 x 10 ⁵	Provided by client
Sterilization test (S. typhimurium)	CFU/mL	30 min	3.3 x 10 ⁴ (96.6%)	Provided by client
Sterilization test (S. typhimurium)	CFU/mL	2 hr	5.5 x 10 ² (99.9%)	Provided by client
Sterilization test (S. typhimurium)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client

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Name of sample: Silver ion sterilizer

Test Results				
Test item	Unit	Sample category	Result	Test method
Sterilization test (P.aeruginosa)	CFU/mL	Initial	8.1 x 10 ⁵	Provided by client
Sterilization test (P.aeruginosa)	CFU/mL	30 min	4.7 x 10 ⁵ (42.0%)	Provided by client
Sterilization test (P.aeruginosa)	CFU/mL	2 hr	5.9 x 10 ² (99.9%)	Provided by client
Sterilization test (P.aeruginosa)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client
Sterilization test (S. flexneri)	CFU/mL	Initial	4.5 x 10 ⁵	Provided by client
Sterilization test (S. flexneri)	CFU/mL	30 min	3.3 x 10 ⁵ (26.7%)	Provided by client
Sterilization test (S. flexneri)	CFU/mL	2 hr	1.8 x 10 ⁵ (60.0%)	Provided by client
Sterilization test (S. flexneri)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client
Sterilization test (B. cereus)	CFU/mL	Initial	6.0 x 10 ⁵	Provided by client
Sterilization test (B. cereus)	CFU/mL	30 min	5.2 x 10 ⁴ (91.3%)	Provided by client
Sterilization test (B. cereus)	CFU/mL	2 hr	6.0 x 10 ³ (99.0%)	Provided by client
Sterilization test (B. cereus)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client

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 Address: 1 Yeonsedaegil, Heungeop-myeon, Wonju-si, Gangwon-do, Korea
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Name of sample: Silver ion sterilizer

Test Results				
Test item	Unit	Sample category	Result	Test method
Sterilization test (B. subtilis)	CFU/mL	Initial	1.1 x 10 ⁵	Provided by client
Sterilization test (B. subtilis)	CFU/mL	30 min	1.2 x 10 ⁴ (89.1%)	Provided by client
Sterilization test (B. subtilis)	CFU/mL	2 hr	1.1 x 10 ² (99.9%)	Provided by client
Sterilization test (B. subtilis)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client
Sterilization test (L. monocytogenes)	CFU/mL	Initial	1.6 x 10 ⁵	Provided by client
Sterilization test (L. monocytogenes)	CFU/mL	30 min	9.1 x 10 ⁴ (43.1%)	Provided by client
Sterilization test (L. monocytogenes)	CFU/mL	2 hr	9.0 x 10 ³ (94.4%)	Provided by client
Sterilization test (L. monocytogenes)	CFU/mL	24 hr	< 10 (more than 99.9%)	Provided by client

* Test conditions: See report.

** Reduction rate (%) = $\{(A - B)/A\} \times 100$

Where A: Initial bacterial population

B: Bacterial population at certain time point of treatment

*** Test strains: Escherichia coli ATCC 25922

Staphylococcus aureus ATCC 6538

Salmonella typhimurium ATCC 13311

Pseudomonas aeruginosa ATCC 27853

- Continued on next page -

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(304 Disabled Business Incubation Center)

Name of sample: Silver ion sterilizer

Test Results				
Test item	Unit	Sample category	Result	Test method
Shigella flexneri ATCC 12022				
Bacillus cereus ATCC 14579				
Bacillus subtilis ATCC 6633				
Listeria monocytogenes ATCC 19111				

Attachment: Test results

* Purpose: Quality management.

- Notes:
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November 14 2014

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8.2 Image of sample



Silver ion sterilizer